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Mutational Bias for Body Size in Rhabditid Nematodes

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ABSTRACT

Mutational bias is a potentially important agent of evolution, but it is difficult to disentangle the effects of mutation from those of natural selection. Mutation-accumulation experiments, in which mutations are allowed to accumulate at very small population size, thus minimizing the efficiency of natural selection, are the best way to separate the effects of mutation from those of selection. Body size varies greatly among species of nematode in the family rhabditidae; mutational biases are both a potential cause and a consequence of that variation. We report data on the cumulative effects of mutations that affect body size in three species of rhabditid nematode that vary fivefold in adult size. Results are very consistent with previous studies of mutations underlying fitness in the same strains: two strains of *Caenorhabditis briggsae* decline in body size about twice as fast as two strains of *C. elegans*, with a concomitant higher point estimate of the genomic mutation rate; the confamilial *Oscheius myriophila* is intermediate. There is an overall mutational bias, such that mutations reduce size on average, but the bias appears consistent between species. The genetic correlation between mutations that affect size and those underlying fitness is large and positive, on average.

THE importance of mutation to the evolutionary process is universally appreciated by biologists, both in terms of the deleterious effects on fitness (Morgan 1903; Fisher 1930; Haldane 1937; Sturtevant 1937) and as the ultimate source of potentially adaptive genetic variation. It has been recognized for a long time that there is substantial variation in the mutational process at a variety of taxonomic levels, even among genotypes within species (Sturtevant 1937 and references therein; Woodruff *et al.* 1984; Fry 2004b; Baer *et al.* 2005; Ávila *et al.* 2006; Haag-Liautard *et al.* 2007). The factors responsible for that variation are poorly understood, but there are two classes of potential explanations. First, the mutation rate may be primarily a by-product of intrinsic or extrinsic environmental factors, *e.g.*, temperature, metabolic rate, UV exposure, etc. (Martin and Palumbi 1993; Hebert *et al.* 2002; Gillooly *et al.* 2005). Alternatively, the mutation rate may be an evolutionarily optimized property, with either the optimum or the deviation from the optimum varying among taxa (Kimura 1967; Leigh 1973; Kondrashov 1995; Dawson 1998). Elucidating the taxonomic distribution of variation in mutational properties may provide important insights into several disparate areas of evolutionary biology, among them the causes of adaptive radiation (Bjedov *et al.* 2003; Sikorski and Nevo 2005) and cladogenesis (Shpak 2005), the rate of molecular evolution (Martin and Palumbi 1993; Gillooly *et al.* 2005), the nature of selection on modifier loci (Kondrashov 1995), the evolution of genetic architecture underlying the phenotype (Jones *et al.* 2003), and the evolution of mating system and sexual reproduction (Kondrashov 1988, 1995; Keightley and Otto 2006).

Of particular interest to quantitative geneticists is the relationship between the average phenotypic effect of a new mutation and the starting phenotype. If mutational effects are biased, the evolutionary process will be biased from the start (Jones *et al.* 2003) and long-term evolutionary trends may have more to do with mutation and drift than with natural selection (Lande 1975). For example, if new mutations are more likely to decrease size than increase it, all else being equal, an evolutionary decrease in size is more probable. Conversely, if mutational effects are not biased, change in any direction is equally likely and, at least in principle, any phenotype will be eventually achievable. Obviously, mutational effects must ultimately be constrained (*e.g.*, size must be positive and finite) and thus biased at the boundary of the possible phenotypic range, but the range of allowable mutational space is generally not known.

Disentangling the relative contributions of the phenotypic effects of mutation *per se* and natural selection

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to an apparent mutational bias is not straightforward, because all alleles potentially have pleiotropic effects on fitness. For a fanciful but illustrative example, consider the vertebrate head. Adult individuals with two heads are occasionally found in nature; adults with no head never are. Presumably this bias is not due to the greater frequency of mutations for two than zero heads, but rather because mutations that result in no head are invariably lethal early in development.

The confounding of mutational *vs.* selective bias can never be fully overcome, but it can be minimized by allowing mutations to accumulate under conditions in which natural selection is minimized. The method of mutation accumulation (MA) minimizes the effects of natural selection by allowing replicate lines of a highly homozygous genotype to evolve at very small population size; mutations with effects on fitness $s < 1/4N_e$ will accumulate at approximately the neutral rate (Kimura 1962).

To date, most of the relevant data are for traits that are closely associated with fitness and are expected to be under strong directional selection (reviewed in Drake *et al.* 1998; Keightley and Eyre-Walker 1999; Lynch *et al.* 1999). There is a large body of theoretical (*e.g.*, Lande 1975; Turelli 1984; Jones *et al.* 2003; Waxman and Peck 2003) and empirical work concerning the mutational properties of traits that are expected to be under stabilizing selection (especially bristle number in *Drosophila melanogaster*, *e.g.*, Clayton and Robertson 1955; Fry *et al.* 1995; Nuzhdin *et al.* 1995; Mackay and Lyman 1998; García-Dorado *et al.* 2000), but relatively few studies that allow a straightforward *post hoc* comparison of the same trait among taxa, especially metazoans (see Houle *et al.* 1996). To our knowledge no study has been explicitly designed to elucidate the variation in mutational properties among taxa in a trait not expected to be highly correlated with fitness.

Body size in nematodes in the family Rhabditidae provides an ideal opportunity to investigate the issues considered above. There is considerable variation within the family in several components of body size, including absolute adult size and percentage of growth following maturation (Flemming *et al.* 2000). Two studies with MA lines of the N2 strain of *Caenorhabditis elegans* showed that there is a mutational bias, with spontaneous mutations tending to reduce body size (Azevedo *et al.* 2002; Estes *et al.* 2005). Here we report a comparative study of the cumulative effects of spontaneous mutations on body size in three species of nematode in the family Rhabditidae that vary fivefold in maximum adult body volume.

MATERIALS AND METHODS

Systematics and natural history of nematode strains: Justification for choice of species and strains is given in Baer *et al.* (2005). We used two species in the genus Caenorhabditis, *C. elegans* and *C. briggsae*, and the confamilial species *Oscheius myriophila*. All are androdioecious hermaphrodites; androdioecy appears to have evolved independently in these three species (Kiontke *et al.* 2004) Hermaphrodites can outcross to males only (Wood 1988), which are rare in laboratory cultures of all three species (~0.1% in most strains of *C. elegans*). Generation time of all three species at 20° is ~3.5 days, and fecundity is similar in all species. Each species is represented by two strains (iso-hermaphrodite lines): N2 and PB306 in *C. elegans*, HK104 and PB800 in *C. briggsae*, and EM435 and DF5020 in *O. myriophila*. *C. briggsae* and *C. elegans* are believed to have diverged at least 50 MYA (Denver *et al.* 2003), with Caenorhabditis and Oscheius having diverged well before then. Collection information on all strains is available from the Caenorhabditis Genetics Center.

Mutation accumulation: MA protocols employed in this study have been outlined in detail elsewhere (Vassilieva and Lynch 1999; Baer *et al.* 2005). The principle is simple: many replicate lines of a highly inbred stock population are allowed to evolve in the relative absence of natural selection, thereby allowing deleterious mutations to accumulate. Descendant populations are then compared to the ancestral control stock. If the average effect of new mutations is nonzero, the mean phenotype will change over time. Since different lines accumulate different mutations, the variance among lines will increase over time, even if the average mutational effect is zero.

For each of the four Caenorhabditis strains we assayed 68 (of the initial 100) MA lines that had accumulated mutations for 200 generations and 30 ancestral control lines. Fewer MA lines were available for the *O. myriophila* strains due to loss of lines during freezing (DF5020, $n = 47$; EM435, $n = 43$). MA and control lines were randomly assigned to two blocks of equal size; each MA line was present in only one block (*i.e.*, line is nested within block). At the beginning of a block, 34 randomly chosen MA lines from each strain (half of the remaining *O. myriophila* lines) were thawed. A sample of each control population was thawed and 15 worms were chosen to begin replicate lines and allowed to reproduce. Three replicates were started from a single worm and maintained by single-worm transfers for two generations (P1 and P2). Each plate was assigned a random number and was handled only in random numerical order after the first generation. If a worm failed to reproduce during the P1 generation, we started the plate again. A single gravid adult (~96-hr) P3 worm was collected from each P2 parent. The gravid P3 adult was then allowed to lay eggs on a fresh plate for ~2 hr; 72 hr after egg laying, 10 adult worms were collected into microcentrifuge tubes containing a fixative (4% glutaraldehyde buffered with PBS). If a worm did not reproduce during the 2-hr period, another gravid adult was selected from the P2 plate and the process was repeated. From each replicate, 5–10 worms were randomly picked out of the fixative, suspended in PBS buffer, and photographed at 50× magnification. Replacement of worms that failed to reproduce at either the P1 or the egg-laying stage potentially imposes selection. Because size is positively correlated with fitness (see below), differences between MA and control groups would have been underestimated, more so in *C. briggsae* than in the other two species.

Worm measurements: Our measurement protocol follows that of Azevedo *et al.* (2002). Adult worms were photographed using a Leica MZ75 dissecting microscope. Images were captured using a Leica DFC280 camera connected to a computer running the Leica IM50 software (Leica Microsystems Imaging Solutions). Images were imported into the public domain ImageJ software (http://rsb.info.nih.gov/ij/), and individuals were measured by manually adjusting the threshold of the image and automatically tracing animal outlines using

the "Analyze Particles" option within the "Analyze" menu. Area (A) and perimeter (P) were calculated for each individual and used to estimate body volume (S) under the assumption that the worm is cylindrical using the equation $S = \pi\left(P + \sqrt{P^2 - 16A}\right)\left(P - \sqrt{P^2 - 16A}\right)^2/256$ (Azevedo *et al.* 2002; note that a typographical error in the original publication omitted the exponent in the numerator).

DATA ANALYSIS

Differences among groups in the change in mean phenotype: The change in mean phenotype due to the accumulation of new mutations $\Delta\bar{z} = U\bar{a}t$, where U is the genomic mutation rate, $2a$ is the homozygous effect of a mutation, and t is the number of generations of mutation accumulation (Lynch and Walsh 1998, p. 341). The average effect, $\bar{a}$, is typically expressed as a fraction of the starting mean. To allow meaningful comparisons among groups, data were natural log-transformed prior to analysis so that equivalent proportional changes in groups with different control means ($\bar{z}_0$) have equivalent slopes (R_m), where $R_m = (z_t - z_0)/t$. Residuals of log-transformed replicate means were slightly left skewed but not significantly different from normal (Shapiro–Wilks' $W = 0.998$, $P < W = 0.35$); obvious outliers were removed by eye. We first tested for a change in mean body volume in each strain individually with the linear model log(volume) = Generation + Block + Line(Generation × Block) + Error as implemented in SAS v. 9.1 PROC MIXED with Generation (gen) (control, gen 0; MA, gen 200) coded as a class variable (supplemental Table 4 at http://www.genetics.org/supplemental/). Generation was modeled as a fixed effect; block and line were considered random effects. Degrees of freedom were determined by the Satterthwaite approximation for unequal sample size. Six tests require an individual Bonferroni-corrected significance level of $\alpha = 0.05/6 = 0.0083$ to maintain an experimentwide type I error rate of 5%. The slope of the regression of log(volume) on Generation (R_m) and its standard error were calculated using the same model in PROC MIXED with the SOLUTION option and Generation was coded as a continuous variable.

We next fit the full model log(volume) = Block + Generation + Species + Generation × Species + Strain(Species) + Generation × Strain(Species) + Line(Block(Gen(Strain(Species)))) + Error. Generation, Species, and their interaction were modeled as fixed effects; other terms were considered random. Species and the interaction of species with generation were considered fixed because prior results suggested particular hypotheses about relative rates of change (R_m) in the different species, *e.g.*, that *C. briggsae* should change faster than *C. elegans* (Baer *et al.* 2005, 2006). Fixed effects were tested for significance using PROC MIXED with degrees of freedom determined by the Satterthwaite method. The specific effect of interest is the species-by-generation interaction, *i.e.*, whether R_m differs between species (supplemental Table 5 at http://www.genetics.org/supplemental/)? Comparisons between pairs of species were performed similarly (supplemental Table 6 at http://www.genetics.org/supplemental/). Three between-species comparisons require a Bonferroni-corrected $P < 0.05/(1 + 3) = 0.0125$ (the 1 in the sum in the denominator accounts for the test of the full model). Differences between strains within each species were tested for using likelihood-ratio tests with the (random) species-by-generation interaction term included and excluded from the model. Three between-strain comparisons require a Bonferroni-corrected $P < 0.05/(1 + 3 + 3) = 0.007$ to maintain an experimentwide 5% probability of type I error.

The most intuitive measure of the cumulative effects of new mutations is the change in the (untransformed) mean expressed as a proportion of the control mean, $\Delta\bar{z} = R_m/\bar{z}_0$. We estimated $\Delta\bar{z}$ by means of a bootstrap procedure. Details of the procedure are presented in Baer *et al.* (2005). Briefly, lines (control and MA) were sampled with replacement from each assay block. The pseudomean values for MA and control lines were calculated using SAS v. 9.1 PROC MEANS and a pseudovalue of $\Delta\bar{z}$ was determined for each block and averaged over blocks for a final estimate. This procedure was repeated 1000 times; the upper and lower 2.5% of the distribution constitute ~95% confidence limits (Efron and Tibshirani 1993). Groups with nonoverlapping confidence limits (CL) are considered significantly different at the 5% level. This protocol accounts for variation both within and between blocks.

Differences among groups in the mutational variance: The per-generation input of genetic variation from new mutation, V_M, is one-half the among-line component of variance divided by the number of generations of mutation accumulation (Lynch and Walsh 1998, p. 330). This calculation is predicated on the assumption that the among-line component of variance in the ancestral control is zero. Comparisons of mutational variances among groups are complicated by scaling effects (see Houle *et al.* 1996; Fry and Heinsohn 2002; Baer *et al.* 2006) that are not necessarily obviated by log-transformation. Mutational variances are commonly scaled either by the environmental variance [mutational heritability $h_M^2 = V_M/V_E$, where V_E is the environmental (error) variance] or by the control mean [mutational coefficient of variation, $CV_M = [100(V_M)^{1/2}]/z_0$ (Houle *et al.* 1996)]. Both of these measures of mutational variability have potentially serious limitations when used in a comparative MA context. Mutational heritability depends on V_E, so differences among groups in environmental variance, for whatever reason, can potentially provide a misleading picture of the variation actually due to new mutations (Houle 1992; Houle *et al.* 1996). Mutational CVs do not depend on V_E and do account for scaling effects. However, the CV_M does not account for differences among groups in the change

in the mean over time (*i.e.*, differences in R_m), so comparisons of CV_M may become misleading over time if groups differ in R_m. We have previously found that different ways of scaling mutational variances can result in qualitatively different inferences about relative variability of different groups (Baer *et al.* 2005, 2006; see also Fry and Heinsohn 2002). We report several measures of mutational variability: V_M of raw and natural log-transformed data, h^2_M calculated from raw and log-transformed data, and CV_M scaled by the untransformed control mean ($CV_{M,Control}$) and by the untransformed MA mean ($CV_{M,MA}$).

Variance components for each strain/generation combination were calculated from the linear model y = Block + Line + Error using restricted maximum likelihood (REML) as implemented in SAS v. 9.1 PROC MIXED (for V_M) or PROC VARCOMP (h^2_M and CV_M). The EM435 strain of *O. myriophila* was omitted from the analysis because of the nonzero among-line variance of the controls. Standard errors of V_M were estimated by REML using the COVTEST option in PROC MIXED; standard errors of h^2_M and CV_M were estimated by a bootstrap protocol analogous to that for $\Delta\bar{z}$. Lines were resampled with replacement with block structure maintained; standard deviations of the pseudodistribution are approximate standard errors when data are normally distributed (Efron and Tibshirani 1993). Plus/minus two standard deviations corresponded very closely to empirical 95% confidence limits in all cases. We used VARCOMP rather than MIXED for the bootstrap analysis because of much faster run times. Means used in calculations of CV_M were calculated from the same pseudosample as the variance components.

Mutational covariance between body size and fitness: Among-group components of (co)variance were determined for body size (generation 200, this study) and fitness (generation 200, Baer *et al.* 2005) from the linear model y = Trait + Block + Line + Error, where the fixed effect Trait represents log(volume) and log(1 + fitness). "Fitness" is lifetime fecundity, weighted by the probability of survivorship. The among-line component of covariance between traits is the genetic covariance, which in this case is the mutational covariance. Genetic correlations r_G and their standard errors were calculated from the among-line components of (co)variance. The analysis was implemented in SAS v. 9.1 PROC MIXED with the unstructured covariance ("TYPE=UNR") option. If mutational effects on fitness and size are perfectly correlated, the expected genetic correlation is 1. The null hypotheses $r_G = 1$ were tested by constraining the among-line correlation to 1 (0.999 in practice) with a "PARMS" statement and comparing the likelihood to that of the unconstrained model (Fry 2004a, pp. 19–23). We also report the correlation of line means ($r\bar{z}$).

Mutation rate and mutational effects: The mutation rate per diploid genome per generation (U) and average homozygous mutational effect ($\bar{a}$) were estimated by the Bateman–Mukai (BM) method (Lynch and Walsh 1998, pp. 341–343). The BM method estimates two parameters, the (downwardly biased) mutation rate $U_{MIN} = (R_m)^2/V_M$ and the (upwardly biased) average effect $\bar{a}_{MAX} = V_M/R_m$, and requires the restrictive assumption of equal mutational effects. We used R_m and V_M calculated from log-transformed data, which is appropriate if mutational effects are multiplicative across loci. Estimates of $\bar{a}_{MAX}$ are not expressed as proportions of the starting mean because they are calculated on log-transformed data, but differences across taxa are comparable. Standard errors of U_{MIN} and $\bar{a}_{MAX}$ were calculated by the Delta method (Vassilieva and Lynch 1999).

We attempted to calculate U and the distribution of mutational effects by maximum likelihood (ML), as implemented by the mlGenomeU v. 2.08 software of Keightley (http://homepages.ed.ac.uk/eang33/mlgenomeu/mlginstructions.html). The method assumes that mutational effects are gamma distributed and accommodates four parameters: U, $\bar{a}$, the gamma shape parameter β, and the fraction of mutations with positive effects P. Models in which β and P were allowed to vary simultaneously failed to converge in reasonable time. Rather than consider only a subset of plausible parameter space, we do not report the results of the ML analysis. It has recently been brought to our attention that ML analysis of MA data in which positive effects are allowed is greatly facilitated by the presence of multiple generations in the data set (F. Shaw, personal communication; also see Keightley and Bataillon 2000).

RESULTS

Change in the mean: Body volume at maturity decreased over 200 generations of MA in all six strains ($R_m < 0$, $P < 0.0083$ in all cases; Figure 1, Table 1, supplemental Table 4 at http://www.genetics.org/supplemental/). ANOVA revealed significant main effects of species and generation and a significant interaction between the two (supplemental Table 5 at http://www.genetics.org/supplemental/). Averaged over strains, *C. briggsae* declined twice as fast as *C. elegans* ($R_{m,Cbr} < R_{m,Cel}$, $P < 0.001$, supplemental Table 6 at http://www.genetics.org/supplemental/), with *O. myriophila* intermediate. In no case did different strains of the same species differ (data not shown). After 72 hr growth at 20°, the *O. myriophila* were about three times as large as the two Caenorhabditis species, less than the fivefold difference in maximum (96-hr) body volume reported by Flemming *et al.* (2000).

Change in the variance: As we (Baer *et al.* 2006) and others (Houle *et al.* 1996; Fry and Heinsohn 2002) have previously observed, genetic variance, mutational or otherwise, depends crucially on scaling. To illustrate, consider the two strains of *C. elegans*, N2 and PB306. When the genetic variance is scaled relative to the

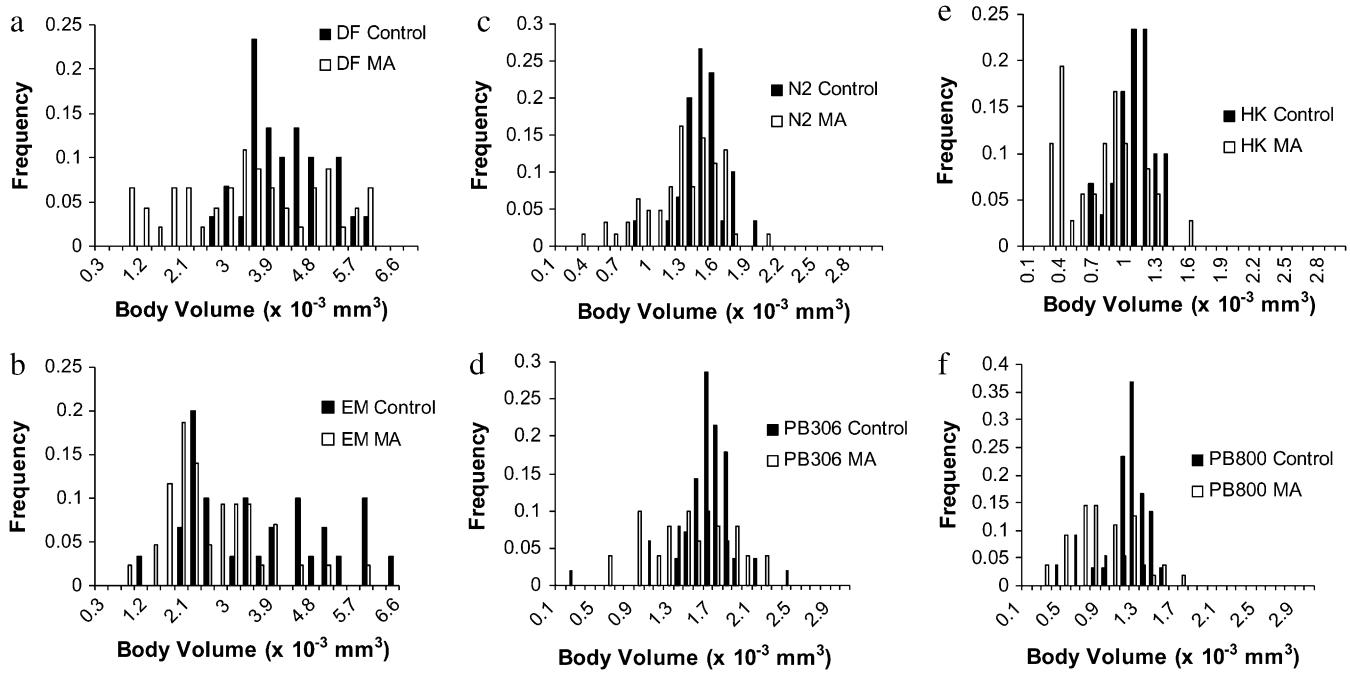

FIGURE 1.—Distributions of mean body volumes in control (solid bars) and mutation-accumulation (open bars) lines for the two strains of *Oscheius myriophila* [DF (a) and EM (b)], two strains of *Caenorhabditis elegans* [N2 (c) and PB306 (d)], and *C. briggsae* [HK (e) and PB800 (f)].

environmental variance (mutational heritability, h^2_M), PB306 is twice as variable as N2, but when the genetic variance is scaled by either the control or the MA mean, the variance in PB306 is only ~20% greater than in N2. The difference between the two measures is due to greater within-line (environmental) variance in PB306. We report the variance scaled in several ways (Table 2), each of which has been widely used in studies of mutational variation. We believe the most meaningful estimate of genetic variability is the coefficient of variation scaled by the MA mean (CV_M, $\bar{z}_{200}$); see CROW (1958) for discussion of the interpretation of the variance scaled in this way. It is worth noting that the "mutational CV" is generally reported as the genetic variance scaled by the control mean in studies in which there is a control and necessarily as the genetic variance scaled by the MA mean in studies in which there is no control. The distinction is important in a comparative context if the mean changes at different rates in different groups and the variance scales with the mean.

Focusing on the MA-scaled CV (CV_M, $\bar{z}_{200}$), the increase in genetic variance is on the order of 1–2%/generation, very consistent with previous studies (HOULE *et al.* 1996; DRAKE *et al.* 1998; AZEVEDO *et al.* 2002). The per-generation increase in genetic variance does not differ significantly between strains or species.

We have omitted estimates of genetic variance for the EM435 strain because the among-line variance in the

TABLE 1

Summary statistics for mean body volume

Strain (species)	DF (*O. myr*)	EM (*O. myr*)	N2 (*C. el*)	PB306 (*C. el*)	HK (*C. br*)	PB800 (*C. br*)
$\bar{z}_0$ ($\times 10^3$)	4.060 (0.449)	3.723 (0.344)	1.374 (0.0.089)	1.604 (0.0.054)	1.060 (0.0.051)	1.116 (0.0.058)
$\bar{z}_{200}$ ($\times 10^3$)	3.348 (0.489)	2.686 (0.260)	1.171 (0.0.093)	1.369 (0.0.073)	0.725 (0.0.067)	0.843 (0.070)
$\log(\bar{z}_0)$	−5.571 (0.117)	−5.756 (0.114)	−6.623 (0.079)	−6.468 (0.026)	−6.895 (0.059)	−6.778 (0.057)
$\log(\bar{z}_{200})$	−5.894 (0.142)	−6.061 (0.096)	−6.837 (0.088)	−6.679 (0.061)	−7.392 (0.098)	−7.194 (0.077)
R_m ($\times 10^3$)	−1.62 (0.47)^{a,b}	−1.53 (0.53)^{a,b}	−1.07 (0.26)^{a}	−1.11 (0.33)^{a}	−2.49 (0.47)^{b}	−2.10 (0.30)^{b}
$\Delta\bar{z}$ ($\times 10^3$)	−0.891	−1.358	−0.746	−0.706	−1.714	−1.409
	(−1.437, −0.294)	(−1.990, −0.635)	(−1.066, −0.388)	(−1.104, −0.268)	(−2.220, −1.157)	(−1.802, −0.988)

Values are means, standard errors are in parentheses. The 95% CL of Δz is shown below the mean. $\bar{z}_0$, mean body volume ($\times 10^2$ mm^3) at generation zero (control); $\bar{z}_{200}$, mean body volume after 200 generations of MA; R_m, regression slope of log(volume) on generation; Δz, per-generation percentage of change in mean (untransformed) body size. *O. myr*, *O. myriophila*; *C. el*, *C. elegans*; *C. br*, *C. briggsae*.

$^{a,b}R_m$ differs significantly between species with different superscripts.

TABLE 2

Summary statistics for mutational (co)variance

Strain (species)	DF (*O. myr*)	EM (*O. myr*)	N2 (*C. el*)	PB306 (*C. el*)	HK (*C. br*)	PB800 (*C. br*)
V_b ($\bar{z}_0 \times 10^9$)	12.69 (35.52)	1552 (680.1)	0.766 (2.69)	3.734 (5.965)	16.82 (10.74)	3.544 (3.503)
V_M ($\bar{z} \times 10^{10}$)	38.06 (11.06)	—	1.839 (0.439)	3.806 (1.103)	1.107 (0.988)	1.721 (0.450)
V_M ($\log\bar{z}$) $\times 10^3$	0.613 (0.180)	—	0.234 (0.060)	0.375 (0.096)	0.389 (0.163)	0.294 (0.088)
h^2_M ($\bar{z}$) $\times 10^3$	2.907 (1.182)	—	3.477 (1.151)	7.601 (3.269)	2.048 (2.961)	2.547 (0.833)
h^2_M ($\log\bar{z}$) $\times 10^3$	3.161 (2.014)	—	3.223 (1.444)	9.593 (7.079)	2.775 (3.030)	2.872 (1.051)
CV_M, $\bar{z}_0$	1.524 (0.233)	—	0.988 (0.124)	1.219 (0.181)	0.994 (0.550)	1.126 (0.156)
CV_M, $\bar{z}_{200}$	1.855 (0.294)	—	1.162 (0.159)	1.420 (0.208)	1.515 (0.795)	1.568 (0.203)
r_G	0.43 (0.21)	0.83 (0.35)	0.70 (0.20)	0.93 (0.07)	0.22 (0.42)	0.64 (0.16)
$r_{\bar{z}}$	0.30	0.37	0.35	0.51	0.18	0.43

Values are strain means; standard errors are in parentheses. V_b ($\bar{z}_0$), among-line variance at generation 0 (control); V_M, mutational variance; h^2_M, mutational heritability; CV_M, $\bar{z}_0$, mutational coefficient of variation, scaled by the control (gen 0) mean; CV_M, $\bar{z}_{200}$, mutational CV scaled by the MA (gen 200) mean; r_G, genetic correlation between log(volume) and log($\bar{W}$); $r_{\bar{z}}$, correlation of line means between log(volume) and log($\bar{W}$). *O. myr*, *O. myriophila*; *C. el*, *C. elegans*; *C. br*, *C. briggsae*.

control stock of that strain was significantly greater than zero (Table 2). The consistent presence of among-line variance in the EM435 control stock suggests that six generations of inbreeding did not render that stock completely homozygous. Inadvertent contamination of the ancestral EM435 cannot be ruled out, but since worms have exceedingly short ranges when not in liquid or on agar media, contamination in worm experiments is almost always due either to mislabeling of plates or tubes, in which case a stock will be homozygous for a different genotype than expected, or to poor handling during high-throughput cryopreservation, at which time worms are in liquid. Ancestral stocks were handled one at a time during cryopreservation, so residual segregating genetic variation, perhaps due to a new mutation that occurred during the six generations of inbreeding, seems more likely than contamination of the EM435 control stock.

Rate and average effects of new mutations: U_{MIN} estimated by BM is "small," on the order of 0.2–2% per generation (Table 3), with average homozygous effects on the order of 0.15–0.3. U_{MIN} does not differ significantly between species, but point estimates of U_{MIN} are larger for *C. briggsae* than for *C. elegans*, consistent with the significantly greater rate of change in the mean in *C. briggsae*. Overall, the BM estimates are quite consistent with the values obtained by Azevedo *et al.* (2002) for the N2 strain of *C. elegans* (Table 3). The point estimates of U_{MIN} for body volume are somewhat smaller than those for fitness in the same strains (Baer *et al.* 2005), which suggests that body size provides a smaller (but not much smaller) mutational target than does fitness, as expected.

Mutational correlation of body size with fitness: Averaged over all six strains, the genetic correlation between mutations for body volume and mutations that affect fitness ($\bar{W}$; Baer *et al.* 2005) is large and positive, on the order of 0.6, although there is considerable variation between strains in the point estimates (Table 2). Genetic correlations did not differ significantly from 1 except in DF5020. The correlation of line means is considerably smaller, on the order of 0.35 averaged over strains.

DISCUSSION

This study was motivated by two considerations. First, as part of an ongoing effort to characterize natural variation in the rate and properties of spontaneous mutation, the objective was to determine the extent to which the conclusions about mutational properties derived from estimates of fitness generalize to a character with a different mutational target and (presumably) mode of selection. We previously determined that the two strains of *C. briggsae* decline in fitness approximately twice as fast as the two strains of *C. elegans*, with the DF5020 strain of *O. myriophila* declining at about the same rate as *C. elegans* (Baer *et al.* 2005). The EM435

TABLE 3

Estimates of mutational parameters

Strain (species)	U_{MIN} ($\times 10^2$)	$\bar{a}_{MAX}$
DF (*O. myr*)	0.45 (0.54)	−0.37
EM (*O. myr*)	—	—
N2 (*C. el*)	0.44 (0.44)	−0.23 (0.15)
N2–KC (*C. el*)	0.06 (0.31)	NA
N2–VL (*C. el*)	0.36 (0.40)	NA
PB306 (*C. el*)	0.32 (0.39)	−0.34 (0.14)
HK (*C. br*)	1.59 (1.37)	−0.16 (0.07)
PB800 (*C. br*)	1.50 (0.96)	−0.14 (0.05)

Estimates of U_{MIN} from additional N2 data sets (Azevedo *et al.* 2002) are included for comparison; note that haploid values of U_{MIN} are reported in the original publication. U_{MIN}, Bateman–Mukai estimate of diploid U (standard error in parentheses); $\bar{a}_{MAX}$, Bateman–Mukai estimate of average homozygous effect (not scaled to the control mean); NA, not applicable. *O. myr*, *O. myriophila*; *C. el*, *C. elegans*; *C. br*, *C. briggsae*.

strain of *O. myriophila* is anomalous because, although the MA lines exhibit an increase in variance in fitness over 200 generations, mean fitness after 200 generations is not significantly different from that at time 0.

Overall, the results from this study are very consistent with our previous findings concerning mutations underlying fitness. On average, the two strains of *C. briggsae* decline in mean body volume twice as fast as the two *C. elegans*, with a concomitantly higher estimated U; the two *O. myriophila* are intermediate between the two Caenorhabditis (Tables 1 and 3). Conversely, the rate of increase of genetic variance was quite similar both within and among species (Table 2).

The second objective of this study was to characterize the relationship between starting phenotype and the direction and magnitude of mutational effects, with the hope of shedding light on the nature of evolutionary biases. Adult *O. myriophila* are larger than either of the Caenorhabditis species. There are three possible explanations: natural selection, genetic drift, and mutation. Given that the two genera diverged on the order of 50 MYA (Denver *et al.* 2003) and given the plentiful input of genetic variation for body size, genetic drift is an obvious possibility. Similarly, given the abundant genetic variation, diversifying selection on body size could clearly have led to the observed difference between these species in body size. Two less orthodox possibilities are (1) there are underlying mutational biases (ultimately inseparable from selective biases) such that mutations that increase body size are more common in *O. myriophila* and/or that mutations that decrease body size are more common in Caenorhabditis or, alternatively, (2) it may be that the nature of the developmental architecture of the worm allows a worm to be so large or small only before any further increase/decrease in size becomes prohibitively deleterious. If the species in question are pushing the limits of the functionally allowable distribution of body size, the result would be a mutational bias such that a greater fraction of allowed mutations decrease size in the large species and increase size in the small species.

The results of this study provide strong evidence for an overall mutational bias: on average, new mutations decrease body size in all six strains. The generally large, positive genetic correlation between size and fitness suggests that, not surprisingly, mutations that decrease size are deleterious. There is no trend for larger species to have smaller average mutational effects (Table 3). It is at least conceivable that the smaller size of *C. briggsae* relative to *C. elegans* is in part due to a higher mutation rate, leading to the occasional fixation of slightly deleterious alleles that reduce size, although that possibility does not extend to the relationship between Caenorhabditis and Oscheius.

Given the importance of body size to the biology of metazoans, it is surprising how few estimates of mutational parameters for body size are available [Keightley and Hill 1992, Caballero *et al.* 1995 (mouse); Toline and Lynch 1994, Lynch *et al.* 1998 (*Daphnia pulex*); Santiago *et al.* 1992, Wayne and Mackay 1998 (*Drosophila melanogaster*); Azevedo *et al.* 2002, Estes *et al.* 2005 (N2 strain of *C. elegans*)]. Estimates of mutational correlations between body size and fitness are available only for *D. melanogaster* and *C. elegans*. Wayne and Mackay (1998) found no significant correlation between MA line means for body size and competitive fitness in *D. melanogaster*. In *C. elegans*, Azevedo *et al.* (2002) reported correlations of line means on the order of 0.2–0.3 between body volume and productivity ($r\bar{z} = 0.32$ for the VL stock of N2, compared to our estimate of $r\bar{z} = 0.35$ for the same strain). Estes *et al.* (2005) reported a genetic correlation between body width and intrinsic rate of increase of $r_G = 0.58$ for the VL stock of N2. Correlations of line means will underestimate the true genetic correlation between traits when the traits are measured on different individuals because the sampling covariance between traits is zero (Table 2); thus, our results appear very consistent with previous studies. Azevedo *et al.* (2002) also performed artificial selection on new mutations for body volume. They observed direct responses to selection for both increased and decreased volume, but they observed no significant correlated response in productivity, which they interpreted as indicative of lack of genetic correlation between size and productivity. However, artificial selection will preferentially fix mutations without deleterious pleiotropic effects on fitness. Moreover, compensatory mutations that increase fitness of mutationally degraded lines are not trivially rare in the N2 strain of *C. elegans* (Estes and Lynch 2003). Given the consistent mutational decline in both body size and fitness and the observed positive genetic correlation between size and fitness in this study and that of Estes *et al.* (2005), we believe that the response to selection was largely due to mutant alleles with atypically nonnegative effects on fitness.

Prior studies with these strains showed that *C. briggsae* declines in fitness due to the cumulative effects of spontaneous mutations at about twice the rate as *C. elegans*, with the DF5020 strain of *O. myriophila* similar to *C. elegans* (Baer *et al.* 2005, 2006). This study shows that that result extends to body size, leading to the conclusion that variation in the mutational properties of these strains is not peculiar to a particular trait but instead appears to be a general property. The strength and direction of the mutational bias–mutations make worms smaller, on average—are similar among genotypes and species. The possibility that the great variation in body size among Rhabditid species may result from fundamentally different mutational architecture (*sensu* Jones *et al.* 2003) is not supported.

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